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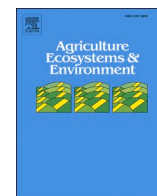
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Emissions of N₂O following field incorporation of leguminous and non-leguminous cover crops

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ABSTRACT

Cover crop (CC) cultivation can reduce N leaching from agricultural fields. It has also recently been proposed as a measure to mitigate climate change due to its potential for increasing soil carbon (C) stocks and reducing soil nitrous oxide (N₂O) emissions during CC growth. However, the input of immature, low C/N ratio plant material to the soil can increase the risk of N₂O emission upon CC termination. In a field trial under humid temperate conditions, N₂O emissions from two leguminous CC species – hairy vetch (*Vicia villosa*) and crimson clover (*Trifolium incarnatum*) – and from two non-leguminous CC species – oilseed radish (*Raphanus sativus*) and winter rye (*Secale cereale*) – were measured in the spring from 24 days before to 54 days after incorporation. The vetch had a significantly higher shoot N concentration and consequently lower C/N ratio than the other three species, but this did not increase N₂O emissions relative to the fallow control. Indeed, oilseed radish was the only cover crop to significantly increase N₂O emissions by 290 g N₂O-N ha⁻¹ during the measurement period relative to the fallow control. These results suggest that factors other than the shoot C/N ratio, such as total biomass, N content or other biochemical parameters may be of greater importance for predicting N₂O emissions from immature cover crop residues.

1. Introduction

Cover crop (CC) cultivation has been suggested as a measure to reduce nutrient leaching from agricultural fields (Abdalla et al., 2019; Thapa et al., 2018; Thorup-Kristensen et al., 2003), but more recently also to mitigate climate change, due to its potential for increasing soil carbon (C) stocks and reducing soil nitrous oxide (N₂O) emissions during the CC growth period (Basche et al., 2014; Kaye and Quemada, 2017). However, while CCs reduce nitrogen (N) availability and therefore may reduce the risk of N losses in the form of nitrate (NO₃) leaching and N₂O emissions during their growth, they may have the opposite effect after their termination. During this period, CCs can increase soil N₂O emissions significantly (Li et al., 2016; Singh et al., 2020). In a recent meta-analysis assessing the effects of crop residue retention on soil N₂O emissions, Abalos et al., (2022) found a strong correlation between crop residue maturity at termination and N₂O emissions. Crops terminated at a vegetative stage, such as CCs, increased N₂O emissions. However, other studies have observed that winter-hardy CCs can reduce late winter/spring N₂O emissions (Foltz et al., 2021; Wagner-Riddle and

Thurtell, 1998), i.e. the impact of CCs on N₂O emissions is ambiguous and not yet fully understood. To avoid offsetting the potential benefits of CC carbon sequestration by increased N₂O emissions, it is important to quantify the magnitude of CC-associated N₂O emissions and elucidate the controlling factors.

Following termination, CC residues may stimulate soil N₂O production by providing substantial inputs of organic C and N to the soil. Depending on the mineralisation/immobilisation rates, this may elevate substrate availability for both nitrification and denitrification processes (Mary et al., 1996). In addition, oxygen (O₂) consumption from microbial respiration may promote O₂ depletion around the decomposing tissue (Mitchell et al., 2013; Mørkved et al., 2006; Zhu et al., 2014), giving rise to zones with anoxic conditions in close proximity to oxic zones (Kravchenko et al., 2017). Such conditions are optimal for coupled nitrification-denitrification (Wrage et al., 2001). Hence, the effect of residue decomposition dynamics on N₂O formation is twofold.

The plant residue C/N ratio has been shown to be strongly negatively correlated to N₂O production and is therefore often used to predict N₂O emissions (Chen et al., 2013; Hu et al., 2019). This aligns with studies

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that have observed higher soil N_2O emissions from leguminous CC residues compared with non-leguminous CC residues (Abdalla et al., 2019; Li et al., 2016; Muhammad et al., 2019). CCs are usually terminated at a young physiological growth stage as the growing season is short in autumn and winter and in regions such as northern Europe characterised by moderate temperatures and restricted light intensity. This often results in rather similar, low C/N ratios, even across distinct CC species such as legumes and non-legumes. Therefore, other CC traits such as biomass production and total residue N or the content of soluble C could be of greater predictive value for N_2O emissions than the C/N ratio *per se* (Abalos et al., 2022; Lashermes et al., 2021). These traits may differ greatly between different CC species.

Although the effect of CCs on N_2O emissions has been studied more frequently in recent years, several field studies compare only one or two species with a control soil, reinforcing the need for studies that assess the effect of different CC species diverging in biomass accumulation and N concentrations on soil N_2O emissions. The aim of the present field trial study was to quantify soil N_2O emissions upon termination of four different CC species, two legumes and two non-legumes, and furthermore to assess the influence of both quantitative and qualitative parameters on N_2O emissions.

2. Material and methods

2.1. Experimental design

A field study was conducted from 11 March to 27 May 2020 in the

Long-Term Nutrient Depletion Trial (LTNDT) field established in 1964 at the University of Copenhagen's research farm in Taastrup, Denmark ($55^\circ 40' \text{ N}$, $12^\circ 17' \text{ E}$; van der Bom et al. 2018). The present study was part of a larger cover-crop field trial running from 2018 to 2020, which has been described in detail by Hansen et al. (2023). The soil was a sandy loam (clay 164 g kg^{-1} , silt 173 g kg^{-1} , fine sand 333 g kg^{-1} , coarse sand 312 g kg^{-1} and organic matter 17 g kg^{-1}). The LTNDT treatment plots used in the current study (M_1P_1 , van der Bom et al. 2018) have received on average $120 \text{ kg NH}_4\text{-N ha}^{-1} \text{ y}^{-1}$, $34 \text{ kg P}^{-1} \text{ y}^{-1}$ and $86 \text{ kg K}^{-1} \text{ y}^{-1}$ in animal slurry since 1996, and also $20 \text{ kg mineral P ha}^{-1} \text{ y}^{-1}$ since 2010. As of 2016, the soil content of Olsen-P and exchangeable K and Mg was 17 , 120 and 90 mg kg^{-1} soil respectively (van der Bom et al. 2018), and thus within moderate to optimal conditions for crop growth. Four different CC species were sown on 20 August 2019 in a randomised block design within each of the four replicated blocks of the LTNDT: the leguminous species hairy vetch (*Vicia villosa*) and crimson clover (*Trifolium incarnatum*) and the non-leguminous species oilseed radish (*Raphanus sativus*) and winter rye (*Secale cereale*). The seeds of hairy vetch were inoculated with *Rhizobium* ($5 \text{ mL inoculant suspension kg}^{-1}$ seeds) a few hours before seeding. The preceding crop was spring barley (*Hordeum vulgare* L. cv. Feedway), sown in spring 2019. Detailed information about pre-crop history, cover-crop species, cultivars and seeding rate is given by Hansen et al. (2023). Each cover crop species was sown in $3 \text{ m} \times 12 \text{ m}$ plots within each of the larger ($20 \text{ m} \times 50 \text{ m}$) LTNDT plots. A plot without a CC was established as a fallow control and harrowed once in the autumn to control weeds. All four CC species survived the

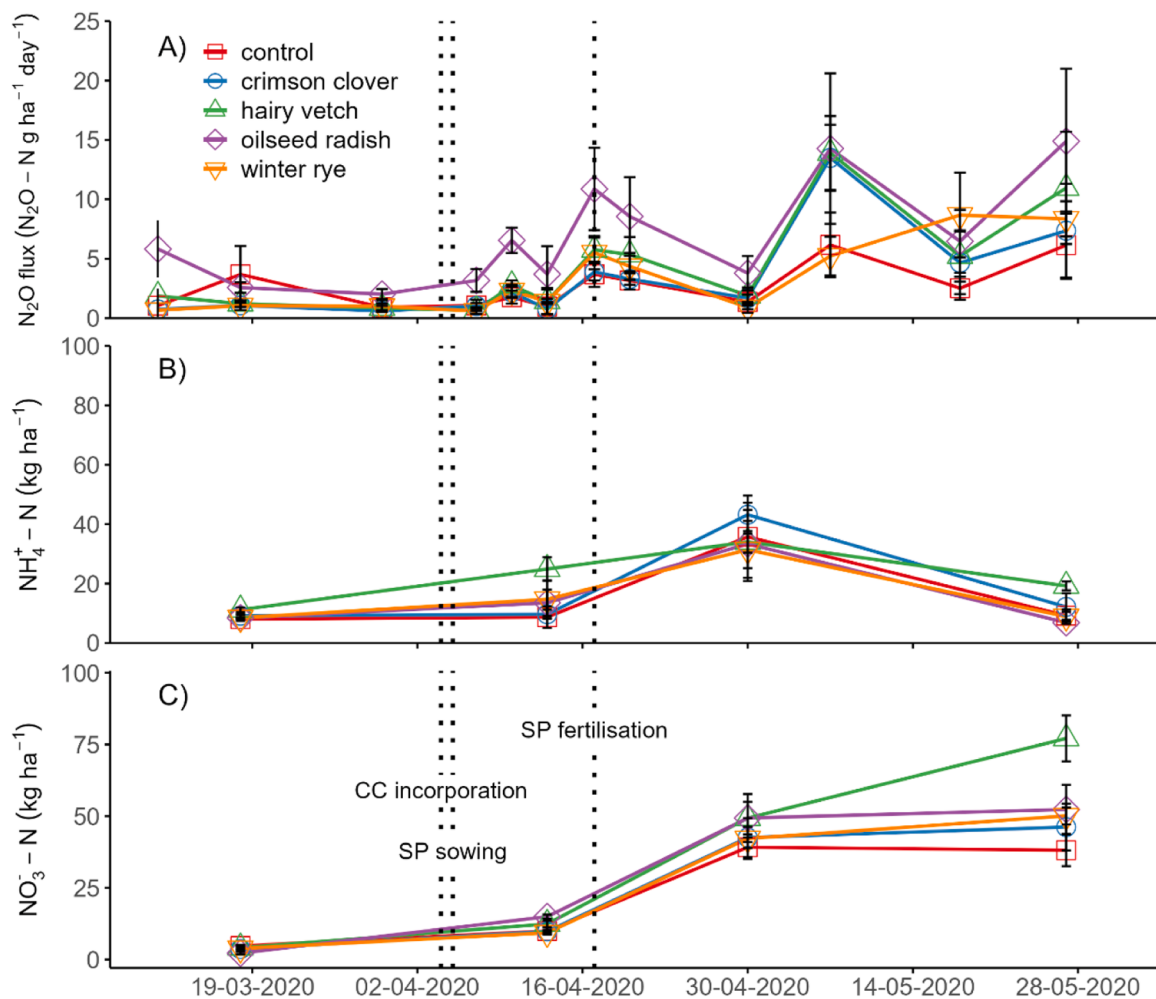


Fig. 1. (A) Daily soil N_2O flux ($\text{g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$), soil concentration of (B) ammonium-N ($\text{kg NH}_4^+\text{-N ha}^{-1}$) and (C) nitrate-N ($\text{kg NO}_3^-\text{-N ha}^{-1}$) in winter rye, oilseed radish, crimson clover, hairy vetch and a fallow control (values are averages with standard error bars ($n=4$)). Dotted vertical lines indicate cover crop (CC) incorporation (4 April 2020), sowing of the succeeding spring barley (SP) (5 April 2020) and its fertilisation (17 April 2020).

winter and were terminated by soil incorporation on 4 April 2020. The late ploughing was due to the wet soil conditions that persisted throughout March. Spring barley (*Hordeum vulgare* L. cv. Feedway) was sown on 5 April, fertilised with 60 kg mineral N ha⁻¹ (as calcium-ammonium-nitrate), 20 kg mineral P ha⁻¹ and 60 kg K ha⁻¹ on 17 April, and harvested on 10 August 2020. Daily precipitation was obtained from a climate station located near to the trial field, while information about soil moisture and temperature (at 0–0.06 m depth) was obtained using data loggers (TMS-4 Soil Measurement System, Tomst, Czech Republic, Wild et al., 2019) placed in the plots with winter rye in each of the four blocks. For information about temperature and precipitation during the growth of the CCs (autumn and winter 2019–2020), see Fig. 1 in Hansen et al. (2023).

2.2. Gas flux measurements

N₂O fluxes were measured 12 times from 11 March (24 days prior to incorporation) to 27 May 2020 (54 days after incorporation). Gas sampling was conducted weekly during most of the experiment; however, the frequency was increased to every second or third day during the period of CC incorporation and spring barley sowing, and fertilization (7–20 April). Static, white-coloured chambers of 0.75 m x 0.75 m x 0.20 m were used for flux measurements, which followed the same sampling procedure as Tariq et al. (2022). Fluxes were calculated in R, version 4.2.3 (R Core Team) using the HMR package (Pedersen et al., 2010), taking air temperature and pressure into account. Cumulated N₂O emissions for the whole period were calculated in R using linear interpolation between the measured fluxes.

2.3. Soil mineral N

Soil samples (0–0.2 m) were collected four times during the gas flux measurement period to determine soil nitrate-N (NO₃-N) and ammonium-N (NH₄⁺-N). Samples were taken in the vicinity of the N₂O chamber system. For each sampling, six 2-cm diameter soil cores (0–20 cm depth) were taken in each plot and pooled into one composite sample, and stored at –18 °C until analysis. The sample was thawed at 4 °C and sieved (< 4 mm). Mineral N was extracted using a representative 10 g subsample of moist soil mixed with 40 mL 1 M potassium chloride (KCl), shaken for 30 min on an end-over-end shaker and filtered through a 2.5-µm filter (Whatman no. 5). The NO₃-N and NH₄⁺-N contents of the extracts were determined using a flow injection analyser (FIAstar 5000 Analyser, FOSS, Denmark).

2.4. Cover crop shoot biomass

The shoot biomass of the CCs was sampled on 18 March 2020 (15 days before incorporation) to determine dry matter accumulation and total C and N contents. The samples were taken using a 0.25-m² metal frame, which was placed randomly in two sampling locations per plot. The plant samples were gently rinsed in tap water and dried at 60 °C for 48 h. Total C and N contents were analysed on finely ground samples using an elemental CN analyser (CNS vario Macro cube, Elementar, Germany).

2.5. Statistical analysis

Statistical analysis was conducted using R, version 4.2.3 (R Core Team). A linear mixed model from the R-package lme4 (Bates et al., 2015) was used to analyse data on N₂O emissions, soil mineral N and CC biomass. Treatments were considered as fixed effects and blocks as random effects. Pairwise comparison using estimated marginal means from the R-package emmeans (Lenth, 2024) were employed to analyse the differences between treatments. For pairwise comparisons, p-values for treatment differences were adjusted using the Tukey method. Significance was determined at $p \leq 0.05$. Prior to analysis, data were

assessed for homogeneity of variance and normality of residual and, if necessary, log transformation was applied. The R codes applied are available in the [Supplementary Material](#).

3. Results

3.1. Environmental conditions

Total cumulative precipitation during the trial period was 72 mm, of which a large part (51 mm) occurred during the second half of the measuring period, i.e. from the end of April and during May (Fig. S1A, [Supplementary Material](#)). Volumetric water content (VWC) ranged from 19 % to 31 % (Fig. S1A). Soil bulk density (BD) was not determined experimentally but, assuming an average BD of 1.62 which has been measured in the same field by Hansen et al. (2024), the measured VWC corresponded to 49–80 % water-filled pore space (WFPS). Soil moisture was at its highest in early March, after which time it gradually decreased during March and April, corresponding to the relatively low precipitation during these months. By the end of April and throughout May, the higher precipitation resulted in higher and fluctuating soil moisture. The average daily air temperatures ranged between 3 and 18 °C and average daily soil temperatures ranged between 1 and 16 °C (Fig. S1B, [Supplementary Material](#)).

3.2. Cover crop shoot biomass

Cover crop shoot dry matter (DM) accumulation ranged from 1260 to 3670 kg DM ha⁻¹ (Table 1). Radish produced the highest biomass, followed by vetch, clover and rye. The N content in shoot biomass ranged between 39 and 116 kg N ha⁻¹ and the order was the same as for DM (radish > vetch > clover > rye). The C/N ratio of the shoot biomass ranged from 8.5 to 12.9 across the CC species, with vetch showing a significantly lower C/N ratio than the other species (Table 1).

3.3. N₂O flux

Soil N₂O fluxes fluctuated greatly, ranging from 1 to 15 g N₂O-N ha⁻¹ day⁻¹ across all treatments and measurements (Fig. 1A). Fluxes were low at the start of the measuring period, but gradually increased after incorporation of the CC biomass, through the sowing and fertilisation of the subsequent spring barley crop and were highest in May when air and soil temperatures had increased, and several precipitation events had occurred. Mean cumulative emissions of N₂O throughout the trial period were highest for radish, followed by vetch, clover, rye and the control (Fig. 2). However, only radish resulted in a significantly greater cumulative flux than the control treatment. The elevated soil N₂O emissions from clover, rye and vetch compared with the control mainly appeared towards the end of the trial (7 May onwards), whereas N₂O emissions from radish were greater than those from the control

Table 1

Dry matter (kg ha⁻¹), total N content (kg ha⁻¹), C/N ratio, C and N concentration (%) of shoot biomass measured on 18 March 2020 (15 days before soil incorporation) in crimson clover, hairy vetch, oilseed radish and winter rye. Values are means, with standard errors in parentheses (n=4). Different letters indicate significant differences between CC species ($p < 0.05$).

	Crimson clover	Hairy vetch	Oilseed radish	Winter rye
Dry matter (kg ha ⁻¹)	1370 (140) ^c	2070 (30) ^b	3670 (290) ^a	1260 (110) ^c
N _{tot} (kg ha ⁻¹)	41 (3) ^c	95 (4) ^b	116 (11) ^a	39 (2) ^c
C/N	11.6 (0.3) ^a	8.5 (0.1) ^b	11.1 (0.5) ^a	12.9 (0.8) ^a
C concentration (%)	35.7 (0.1) ^b	38.6 (0.7) ^a	37.5 (0.1) ^{ab}	40.2 (0.2) ^a
N concentration (%)	3.0 (0.2) ^b	4.6 (0.1) ^a	3.4 (0.2) ^b	3.2 (0.3) ^b

throughout the whole measuring period, except for the measurement on 18 March.

3.4. Soil mineral nitrogen

The soil NH_4^+ -N concentration ranged between 7 and 43 kg ha^{-1} across all treatments and sampling days (Fig. 1B). All treatments except vetch followed the same trend, showing low concentrations ($<15 \text{ kg ha}^{-1}$) on the first, second and fourth samplings, and a peak ranging from 31 to 43 kg NH_4^+ -N ha^{-1} on the third sampling, which was 25 days after incorporation and 13 days after fertilisation. Across all treatments, soil NO_3^- -N concentrations increased gradually during the measuring period to reach the highest level on the fourth and last sampling date (27 May) (Fig. 1C). The treatments showed concentrations in the same range on the first three sampling dates, but vetch resulted in a significantly higher soil NO_3^- -N concentration on the last sampling date, indicating higher net N mineralisation.

4. Discussion

4.1. Higher N_2O emissions from radish compared to legumes

Among the various CC species, legumes have earlier been found to give rise to greater N_2O emissions than non-leguminous species (Abdalla et al., 2019; Muhammad et al., 2019), probably driven by low C/N ratios and consequently relatively rapid decomposition and release of mineral N (Chen et al., 2013). However, in the present study, cumulative N_2O emissions from radish residues exceeded those of the two leguminous species, vetch and clover, despite having a similar or higher C/N ratio to the legumes. Furthermore, radish was the only treatment that significantly increased cumulative emissions compared with the fallow control. The soil N_2O fluxes for clover, rye and vetch only slightly exceeded those of the control (not significantly) due to emission peaks towards the end of the trial period from 30 April, while radish showed increased emissions throughout the entire measuring period (Fig. 1A). This indicates that some mechanisms stimulating N_2O formation occurred in early spring prior to and immediately after the radish was terminated by soil incorporation.

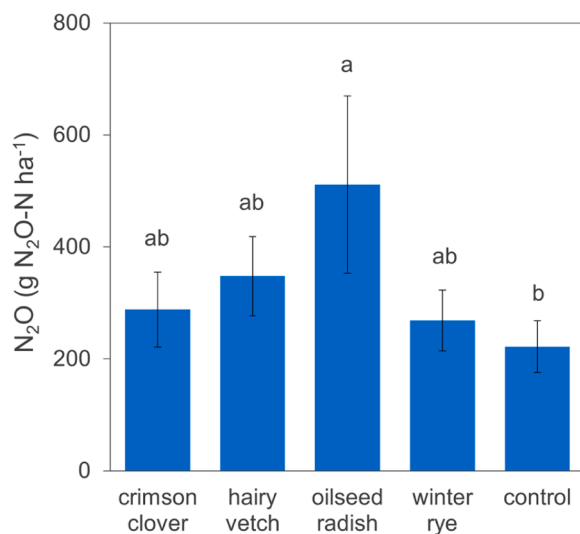


Fig. 2. Mean cumulative emissions of N_2O ($\text{g N}_2\text{O-N ha}^{-1}$) from winter rye, oilseed radish, crimson clover, hairy vetch and a fallow control from 11 March to 27 May 2020 (values are averages with standard error bars ($n=4$)). Different letters represent significant differences ($p \leq 0.05$).

4.2. Effects of cover crop shoot characteristics on N_2O emissions

There are several possible explanations for the higher and earlier N_2O emissions from radish. First, although the N concentration of radish was similar to clover and significantly lower than vetch, it contained the highest amount of N due to its high shoot biomass production (Table 1). This corresponds well with studies identifying N returned with residues as a highly predictive variable for N_2O emissions (Abalos et al., 2022). A high content of N in residues increases the potential for N mineralisation, nitrification and thus NO_3^- availability for denitrification. However, this was not reflected in a higher content of mineral N in the soil under radish (Fig. 1B-C), probably because the NO_3^- was denitrified, or immobilised due to the substantial input of radish residue biomass.

The finding that the N_2O emissions from radish exceeded those of a legume with a lower C/N ratio is in line with other studies. Li et al., (2015) observed significantly higher N_2O emissions from radish compared to vetch. However, in their study radish and vetch produced similar amounts of shoot biomass, but vetch a significantly higher total N content. This suggests that N content alone could not explain the elevated N_2O emissions from radish. In their study, the radish was terminated by climatic conditions during winter, whereas in the current study, it overwintered and was terminated by spring tillage. Despite the differing termination methods, both studies showed strikingly similar N_2O emission patterns for radish, with emission peaks occurring shortly after plant death or termination.

Another explanation for the higher emissions from radish may be that it contains more easily degradable organic compounds than vetch. Hu et al., (2018) reported neutral and acid detergent fiber as well as lignin fractions in immature radish shoots and roots corresponding to a content of 66 % and 40 % neutral detergent solubles (NDS) respectively, and for the roots as much as 44 % cellulose. These values are clearly at the higher end when compared to comprehensive datasets of crop residues from multiple plant species and crop types collected across European countries (Jensen et al., 2005; Thiébeau et al., 2021). Indeed, some studies have found the content of soluble vs. structural compounds to be better predictors of crop residue-induced N_2O emissions than the residue C/N ratio (Abalos et al., 2022; Lashermes et al., 2021). Organic C acts as an electron donor for bacterial and fungal denitrifiers and stimulates O_2 consumption around the plant residues, which creates favourable conditions for denitrification and can constitute a hotspot for N_2O formation (Groffman et al., 2009; Wagner-Riddle et al., 2020). Plant residue hotspots have been shown to be responsible for a large part of N_2O emissions from soils (Parkin, 1987). The fact that the higher emissions from radish mainly occurred prior to and soon after incorporation, at a time when the mineral N content in the soil was relatively low, indicates that the amount of easily degradable organic C plays an important role, and perhaps that the microbial processes responsible for both N mineralisation and denitrification mainly occur in and around the decaying radish plants, which at this developmental stage has a distinct taproot, full of easily degradable C. Oilseed radish is a deep-rooted CC species (Thorup-Kristensen, 2006), however the vast majority of the root biomass is located in the taproot storage organ near the soil surface, in contrast to rye and vetch (Engedal et al., 2023; Hudek et al., 2022), which may have provided more substrate in the topsoil where microbial activity is highest (Fierer and Schimel, 2002), both prior to and after incorporation. Lövgren (2022) studied whether the removal of radish shoot or shoot and taproot biomass could mitigate N_2O emissions. They found that removal of the whole plant (shoot and taproot) was necessary to decrease N_2O emissions significantly, emphasizing the critical role of radish root biomass in these emissions. More research is required to explore how CC roots contribute to N_2O emissions.

The two legumes and the rye only increased N_2O emissions slightly and non-significantly compared with the control. While rye and clover produced a similar low DM and N yield, which can explain the low emissions, vetch yielded a significantly higher DM, N content and lower C/N ratio, but this did not translate into significantly higher N_2O

emissions. However, as discussed above, the content of easily degradable C and the quantity, quality and distribution of root biomass may be of greater importance for N₂O formation than the DM and N content of the shoot biomass, which may explain the small, non-significant differences in the emission pattern between the three species.

4.3. Implications for cover crop species selection to maximize agronomic and environmental services

Cover crops are grown for multiple environmental and agronomic purposes such as reducing N-leaching, providing N or at least avoiding pre-emptive competition for the succeeding cash crop (Thorup-Kristensen et al., 2003), and as recently suggested to increase soil organic C (SOC) sequestration (Kaye and Quemada, 2017; Poeplau and Don, 2015). Hansen et al. (2023) measured the grain yield of the succeeding spring barley in the current field experiment and found that only vetch significantly increased the N content of the grain (Hansen et al., 2023, Fig. 8) By improving the N yield of the succeeding cash crop without elevating N₂O emissions, vetch performed well in terms of the desired agronomic and environmental parameters. However, with its rather slow growth and shallow root system, vetch is less efficient at reducing N leaching compared to fast-growing, deep-rooted species, as radish (Elhakeem et al., 2023; Thorup-Kristensen, 2001). Furthermore, radish produced significantly and substantially higher DM and thus C input to the soil than vetch, which increases the potential for SOC sequestration. Reduced N leaching and increased C input could potentially offset the increased N₂O emission from the radish cover crop. To maximize the benefits of the two cover crop species, a viable option could be to cultivate a species mixture as shown by Elhakeem et al. (2023).

5. Conclusions

In this study on the effect of two leguminous (crimson clover and hairy vetch) and two non-leguminous (oilseed radish and winter rye) cover crops on N₂O emissions upon spring tillage on a sandy loam soil, only oilseed radish significantly increased N₂O emissions compared with fallow soil, despite the oilseed radish having a lower shoot N concentration and higher C/N ratio than the leguminous cover crop vetch. This suggests that other factors such as the content of total N or easily degradable C of the CC biomass and the quantity, composition and distribution of the root biomass may be of greater importance for N₂O formation from CC residues. Future studies should measure CC biochemical composition and root parameters to investigate the influence of these traits on N₂O formation to improve the mechanistic understanding of CC induced N₂O emissions.

CRedit authorship contribution statement

Lars Stoumann Jensen: Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Sander Bruun:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Tine Engedal:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Maria Skovgaard Andersen:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Veronika Hansen:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109335.

Data availability

Data will be made available on request.

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